The oligonucleotides attached to the substrate have a sequence complementary to a first portion of the sequence of a nucleic acid to be detected. The nucleic acid is contacted with the substrate under conditions effective to allow hybridization of the oligonucleotides on the substrate with the nucleic acid. In this manner the nucleic acid becomes bound to the substrate. Any unbound nucleic acid is preferably washed from the substrate before adding nanoparticle-oligonucleotide conjugates.

Next, the nucleic acid bound to the substrate is contacted with a first type of nanoparticles having oligonucleotides attached thereto. The oligonucleotides have a sequence complementary to a second portion of the sequence of the nucleic acid, and the contacting takes place under conditions effective to allow hybridization of the oligonucleotides on the nanoparticles with the nucleic acid. In this manner the first type of nanoparticles become bound to the substrate. After the nanoparticle-oligonucleotide conjugates are bound to the substrate, the substrate is washed to remove any unbound nanoparticle-oligonucleotide conjugates and nucleic acid.

The oligonucleotides on the first type of nanoparticles may all have the same sequence or may have different sequences that hybridize with different portions of the nucleic acid to be detected. When oligonucleotides having different sequences are used, each nanoparticle may have all of the different oligonucleotides attached to it or, preferably, the different oligonucleotides are attached to different nanoparticles. Figure 17 illustrates the use of nanoparticle-oligonucleotide conjugates designed to hybridize to multiple portions of a nucleic acid. Alternatively, the oligonucleotides on each of the first type of nanoparticles may have a plurality of different sequences, at least one of which must hybridize with a portion of the nucleic acid to be detected (see Figure 25B).

Finally, the first type of nanoparticle-oligonucleotide conjugates bound to the substrate is contacted with a second type of nanoparticles having oligonucleotides attached thereto. These oligonucleotides have a sequence complementary to at least a portion of the sequence(s) of the oligonucleotides attached to the first type of nanoparticles, and the contacting takes place under conditions effective to allow hybridization of the

oligonucleotides on the first type of nanoparticles with those on the second type of nanoparticles. After the nanoparticles are bound, the substrate is preferably washed to remove any unbound nanoparticle-oligonucleotide conjugates.

The combination of hybridizations produces a detectable change. The detectable changes are the same as those described above, except that the multiple hybridizations result in an amplification of the detectable change. In particular, since each of the first type of nanoparticles has multiple oligonucleotides (having the same or different sequences) attached to it, each of the first type of nanoparticle-oligonucleotide conjugates can hybridize to a plurality of the second type of nanoparticle-oligonucleotide conjugates. Also, the first type of nanoparticle-oligonucleotide conjugates may be hybridized to more than one portion of the nucleic acid to be detected. The amplification provided by the multiple hybridizations may make the change detectable for the first time or may increase the magnitude of the detectable change. This amplification increases the sensitivity of the assay, allowing for detection of small amounts of nucleic acid.

If desired, additional layers of nanoparticles can be built up by successive additions of the first and second types of nanoparticle-oligonucleotide conjugates. In this way, the number of nanoparticles immobilized per molecule of target nucleic acid can be further increased with a corresponding increase in intensity of the signal.

Also, instead of using first and second types of nanoparticle-oligonucleotide conjugates designed to hybridize to each other directly, nanoparticles bearing oligonucleotides that would serve to bind the nanoparticles together as a consequence of hybridization with binding oligonucleotides could be used.

Methods of making the nanoparticles and the oligonucleotides and of attaching the oligonucleotides to the nanoparticles are described above. The hybridization conditions are well known in the art and can be readily optimized for the particular system employed (see above).

An example of this method of detecting nucleic acid (analyte DNA) is illustrated in Figure 13A. As shown in that Figure, the combination of hybridizations produces dark areas

where nanoparticle aggregates are linked to the substrate by analyte DNA. These dark areas may be readily observed with the naked eye using ambient light, preferably viewing the substrate against a white background. As can be readily seen from Figure 13A, this method provides a means of amplifying a detectable change.

Another example of this method of detecting nucleic acid is illustrated in Figure 25B. As in the example illustrated in Figure 13A, the combination of hybridizations produces dark areas where nanoparticle aggregates are linked to the substrate by analyte DNA which can be observed with the naked eye.

In another embodiment, nanoparticles are attached to the substrate. Nanoparticles can be attached to substrates as described in, *e.g.*, Grabar et al., *Analyt. Chem.*, **67**, 73-743 (1995); Bethell et al., *J. Electroanal. Chem.*, **409**, 137 (1996); Bar et al., *Langmuir*, **12**, 1172 (1996); Colvin et al., *J. Am. Chem. Soc.*, **114**, 5221 (1992).

After the nanoparticles are attached to the substrate, oligonucleotides are attached to the nanoparticles. This may be accomplished in the same manner described above for the attachment of oligonucleotides to nanoparticles in solution. The oligonucleotides attached to the nanoparticles have a sequence complementary to a first portion of the sequence of a nucleic acid.

The substrate is contacted with the nucleic acid under conditions effective to allow hybridization of the oligonucleotides on the nanoparticles with the nucleic acid. In this manner the nucleic acid becomes bound to the substrate. Unbound nucleic acid is preferably washed from the substrate prior to adding further nanoparticle-oligonucleotide conjugates.

Then, a second type of nanoparticles having oligonucleotides attached thereto is provided. These oligonucleotides have a sequence complementary to a second portion of the sequence of the nucleic acid, and the nucleic acid bound to the substrate is contacted with the second type of nanoparticle-oligonucleotide conjugates under conditions effective to allow hybridization of the oligonucleotides on the second type of nanoparticle-oligonucleotide conjugates with the nucleic acid. In this manner, the second type of nanoparticle-oligonucleotide conjugates becomes bound to the substrate. After the